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Influence of Mycorrhizal Inoculation Treatments on Native Tree and Shrub Survival in a Floodplain, Flathead Indian Reservation

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INFLUENCE OF MYCORRHIZAL INOCULATION TREATMENTS ON NATIVE TREE AND SHRUB BIOMASS AND SURVIVAL IN A FLOODPLAIN, FLATHEAD INDIAN RESERVATION

By

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Influence of Mycorrhizal Inoculation Treatments on Native Tree and Shrub Biomass and Survival in a Floodplain, Flathead Indian Reservation

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Mycorrhizal inoculum treatments of two marketed products, Mycopak and Biogrow, were tested against forest soil, and a control treatment on biomass and survival of *Populus tremuloides, Salix bebbiana, Populus trichocarpa*, and *Cornus stolonifera* during the summer of 2003 and 2004. Because these seedlings are difficult to establish on cobbled riparian floodplains, the species and treatments were tested on two Jocko River floodplains. The Powell site was plowed in the spring of 2003 prior to planting, and the Stranahan site remained unplowed but treated with the broad leaf herbicide 2,4-D amine, in the fall of 2002. Seedlings were planted in the spring of 2003 in randomized blocks in a factorial design with four replications on each floodplain, 50 seedlings per plot. POPTRE and POPTRI were found to have strong linear correlations between ocular estimates of biomass and actual biomass ($r=0.87$ and 0.93 respectively). In 2003 and 2004 estimated biomass of POPTRE was greater in the forest soil treatment on Stranahan and Biogrow treatment on the Powell site ($p<.005$). Estimated biomass of POPTRI was greater with Biogrow in 2003 on both sites and in 2004 on Powell ($p< 0.005$). Actual biomass was tested for all species ($n=339$) and an interaction between site and replication ($p<0.001$) significantly affected biomass. In 2003 and 2004 percent of AM hyphal inoculation was significantly affected by site ($p<0.05$) and species ($p<0.001$). In 2003, seedlings showed greater incidence of AM than EM (septate) hyphal inoculation. From 2003 to 2004, seedlings showed a greater percent increase in EM hyphae over AM hyphae in the root. The greatest number of POPTRE, SALBEB, and CORSTO seedlings surviving in 2003 were in the Biogrow treatment; however there was a significant interaction between treatment and site ($p<0.05$) with greater survival on Powell.

A 180-day greenhouse study mimicked the field trial in regards to seedlings and treatments. Mycorrhizal treatment had a significant affect ($p<0.001$) on biomass, while species ($p<0.05$) and an interaction between species and treatment ($p<0.05$) affected the percent AM fungi hyphal inoculation. Incidence of EM hyphae was greater across nearly all species and treatments compared to AM Hyphae.

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INTRODUCTION

Wooded riparian floodplains provide food, fiber, big game connectivity, recreational amenities, soil stabilization, biological diversity, and species richness. Deforestation of riparian floodplains, often associated with crop or hay production for "improved" land uses, result in dramatic changes of vegetation and natural process. A change in species composition may reduce the capacity for self-repair over time and change the direction of succession. Rehabilitation of these damaged wildlands includes repair of the natural ecosystem processes to help initiate autogenic repair within the system for long-term sustainability (Whisenant 2001). When planning landscape restoration and rehabilitation of natural landscapes, ecosystem processes must be incorporated to help sustain cycles of nutrients, hydrology, and energy. Repair efforts implemented at the landscape level should incorporate beneficial structural and functional relationships between interacting landscape elements (Whisenant 2001). Mycorrhizal fungi are one of the elements to consider when planning restoration of damaged landscapes to incorporate structure and function of the restoration site.

Approximately 90% of all plant species form mycorrhizal relationships (Allen 1993). Mycorrhizas are highly evolved, mutualistic associations between soil fungi and plant roots and help sustain ecosystem processes. The two most common mycorrhizal associations are endomycorrhizas (also called vesicular arbuscular mycorrhizae) (AM) and ectomycorrhizas (EM). EM fungi occur on about ten percent of the world's flora and trees, including Pinaceae and Salicaceae, both typical in Montana near low elevation floodplains. More than 2,100 species of fungi form ectomycorrhizae on 2,000 species of forest woody plants. AM fungi

thrive in grasslands (van der Heijden and Sanders 2003). Over 90 percent of the 100,000 species of vascular plants in the world become inoculated by AM fungi (Marx 2001).

Over the years, many woody species have been removed from floodplains for crops and access to stream channels. Harvesting host trees eliminates the photosynthate source for dependent ectomycorrhizal fungi (Whisenant 2001). Following a change in plant composition from fire or deforestation, a decline in site mycorrhizae may drive the site to a non-mycorrhizal community of opportunistic, ruderal species (Whisenant 2001, Brundrett, et al. 1996, Perry and Amaranthus 1990). The Stranahan and Powell research sites are two floodplain locations on the Jocko River in Montana where the dominant shrub and tree communities were removed for planting crops and non-native pasture grasses for agricultural production.

Microbial composition of clear-cut soils occupied by grasses differs from that of forest soils. These changes in above ground community composition alter the quality and quantity of root exudates utilized by mycorrhizae (Amaranthus 1990). Under an open canopy, over time, many grass and shrub species will form AM fungi associations incompatible with most forest species. On these sites that become long dominated by AM species, the EM fungi will gradually diminish and the soil microbial complex associated with EM fungi can be reduced (Amaranthus 1990). When planting into an AM grassland, survival and growth of numerous native tree and shrub species can be improved by inoculation with EM fungi specific to the species planted (Amaranthus 1990, Allen 1993, Chen et al. 2000).

Restoration in cobbled, grassy floodplains has proven difficult with juvenile shrub and tree species along the Jocko River (Kloetzel 2001). Many studies using microbial inoculum address the increase of survival, biomass, and root growth in controlled glasshouse experiments (van

der Heijden and Sanders 2003). Fewer studies address microbial inoculum in field plantings for any extended period of time due to the number of uncontrolled environmental factors in nature, including soil food webs, herbivory, trampling, flooding, drought, temperature, competition, disease, and nutrient availability. These factors greatly influence plant survival, biomass, and vigor, and cannot be experimentally controlled at the landscape scale. To date, mycorrhizal field studies have included soil sterilization or the use of fungicides in mycorrhizal fungi studies(van der Heijden and Sanders 2003). These techniques are too extreme for landscape restoration and rehabilitation and may cause more damage than good as a rehabilitation strategy. However, without sterilization techniques, saprobes and pathogens may be introduced in forest and field soil additions to planting sites in an attempt to add mutualists (Schwartz et al. 2006).

Numerous products are marketed for reforestation to increase seedling survival and biomass when transplanting on disturbed soils or soils that may not contain host specific mycorrhizal fungi. Seedlings can also be inoculated with fresh forest soil inoculum propagules from established populations. Propagules may include pieces of root containing mycorrhizal hyphae, spores, or dry mycelium of desired organisms (Perry and Amaranthus, 1990). When available near the restoration site, these soils may, on a small scale, provide host specific mycorrhizal inoculum for planting native trees and shrubs. Whole soil contains a whole host of mycorrhizal species specific to its host plant. Perry and Amaranthus (1990) suggest gathering soil from near the roots of a healthy host plant that supports beneficial organisms. The host plant may or may not be the same species used in a reclamation project but it is safer to use forest root soil from the same species. Soil should be collected from younger trees due to the successional changes in mycorrhizal fungi over time as trees mature.

The literature is limited on the use of fresh soil with inoculum potential for microcosm or field planting. Fresh soil is not used in research because of the possibility of importing disease causing organisms and is therefore sterilized. However, in Berman and Bledsoe (1998) added riparian forest soil, sterilized forest soil, and agriculture soil to acorn planting holes in an agricultural field where it was presumed the agricultural field would be low or lacking in EM fungi. They found EM roots in all three treatments after ten months. Shoot biomass was greatest in the forest soil treatment. In a replicated greenhouse study they found both the percent mycorrhizal infection and mycorrhizal diversity were increased by using the forest and woodland soils.

The experiments with forest soil in 1990 (Perry and Amaranthus) and 1998 (Berman and Bledsoe), and the words of Read (2003), suggest we have a long way to go to understanding the impacts of mycorrhizae on species composition and dynamics. There are volumes of precise data outlining mycorrhizal functions under simplified conditions with relatively little data towards understanding ecosystem level processes. Incorporating forest soil in reclamation, rehabilitation, or restoration of damaged landscapes only requires a little planning, a little intuition, and a little common sense.

LITERATURE REVIEW

Mycorrhizal fungi are found in every terrestrial ecosystem and represent one of the largest biomass components of those ecosystems (Pierzynski et al. 2000). Allen (1993) defined mycorrhiza as "… a mutualistic symbiosis between plant and fungus localized in a root or root-like structure in which energy moves primarily from plant to fungus and inorganic resources move from fungus to plant." Mycorrhizal fungi help the plant acquire soil nutrients and water in exchange for photosynthetic carbon products (Harley 1971, Pierzynski et al. 2000, Wardle 2002). Aboveground growth and biomass of plants depend largely upon the soil processes belowground that provide nutrients to plant communities (Pierzynski et al. 2000, Wardle 2002).

Grasses maintain an endomycorrhizal (AM) relationship with fungi, while numerous trees and shrubs maintain an ectomycorrhizal (EM) relationship (van der Heijden and Sanders 2003). Numerous articles written on the study of mycorrhizae in establishing plants in previously unoccupied habitats have demonstrated the importance of mycorrhizae to plant survival (Allen 1993). Specifically, planting trees and shrubs into grasslands previously unoccupied by tree species may necessitate incorporating inoculum with ectomycorrhizal propagules different than those existing in the grassland (Amaranthus 1990, Allen 1993, Chen et al. 2000). This includes the return of native trees and shrubs into sites where they have been absent for an extended time.

Early attempts to establish EM trees in grassland habitats (dominated by AM) were not successful until the introduction of the EM fungal species (Allen 1993). Chen et al. 2000 inoculated *Eucalyptus* seedlings with ectomycorrhizal spores when planting in a community

lacking in abundant EM fungi and found that high growth rates were attained with EM fungi and results with AM fungi in that study were variable.

Berman and Bledsoe (1998) tested the addition of forest soil from valley oak riparian areas against agricultural soils and steam-sterilized forest soil. Treatments were applied in an experimental agricultural field lacking in EM fungi. In this study they found mycorrhizal infection, fine root biomass, and shoot biomass were greatest in the forest soil treatment. Shoot biomass was least in the agricultural soil and intermediate grown in sterilized soil. Factorial greenhouse experiments were conducted by Rowe et al (2007) to test field soil, autoclaved field soil, and commercial inoculum. All late successional plant species responded positively to field inoculum while early successional plant species responded negatively. The early successional plants tended to grow more with the field soil and commercial inoculum mix, indicating a possible successional species shift in the mycorrhizal fungus over time.

One of the most interesting mycorrhizal interactions exist on a tripartite system where a single host plant forms both EM and AM associations, including *Populus* and *Salix* (Allen 1993, Brundrett et al 1996, Lodge 2000) both in the family Salicaceae. Initial colonization are AM with successional EM fungal colonization after AM are active (Allen 1993). AM fungi cannot enter the young portion of the root once the EM hyphae form a hyphal mantel around the apical root tip. The EM hyphae extend into the root only to the limit of the epidermal cell and the AM hyphae occupy the inner cortical cell (Chilvers 1987). Because the EM fungi confine themselves into the root cap, the existing AM mycorrhizal fungi can continue to extend through the inner tissues during root growth perpetuating a dual AM/EM system. (Chilvers

1987). Fungal hyphae also extend along the surface area of the root and increases the plant's acquisition of nutrients beyond the depletion zone created by roots. (Allen 1993, Wardle 2002).

For an extended discussion on soil, nutrient acquisition, and mycorrhizae see Appendix 1.

OBJECTIVES

The overall objective of this study was to determine if tree and shrub biomass and survival could be increased by mycorrhizal inoculation on riparian floodplain sites. Given the lack of ectomycorrhizae in grasslands dominated by endomycorrhizae, native soil was tested against two products with both types of inoculum to increase survival and biomass of *Populus tremuloides* (Quaking aspen), *Salix bebbiana*, (Bebb's willow), *Populus trichocarpa* (Black cottonwood), and *Cornus sericea* (Red-osier dogwood). Specifically, the study objectives were:

1) To determine if two marketed mycorrhizal inoculums or a native soil would increase seedling survival and biomass of *Populus tremuloides, Salix bebbiana, Populus trichocarpa*, and *Cornus sericea* compared to controls; and

2) To determine if forest soil collected below a young host plant for each species at or near the planting sites would provide a greater mycorrhizal inoculation percent in comparison with mycorrhizal inoculums on the market.

STUDY AREA

Research was conducted on two riparian flood plains of the Jocko River, western Montana. These sites will be referred to as the Stranahan and Powell sites. The Jocko Valley is about twelve miles by two miles wide and abuts the south end of Mission Valley where the Jocko River continues to its confluence with the Flathead River (Alt 2001). Land ownership along the Jocko River floodplain is a checkerboard of private, state, federal, and tribal ownership with a variety of land use, including agriculture, ranching, recreation, and wildlife management. The Confederated Salish and Kootenai Tribes of the Flathead Indian Reservation (CSKT) purchased both research sites in 2002 and removed them from agricultural production to manage for fisheries, wildlife habitat, and recreation.

CSKT plans to re-incorporate native vegetation throughout the floodplain to meet its fisheries and wildlife habitat objectives for connectivity across the landscape. At the time of purchase, both sites supported an array of nonnative pasture grasses and noxious and weedy forbs. The dominant, exterior tree canopy includes *Populus trichocarpa*, *P. tremuloides,* and *Pinus Ponderosa* (Ponderosa pine), with a lower native shrub community still in place along the exterior boundaries, including *Symphoricarpos albus* (Snowberry), *Amelanchier alnifolia* (Serviceberry), *Rosa woodsii* (Woods rose), *Cornus stolonifera* (Red osier dogwood), and *Salix bebbiana* (Bebb's willow). These sites are frequented by white tail and mule deer, elk, black bear, and grizzly bear.

The 20-acre Stranahan research site is at the south end of the Mission Valley between the Jocko River and Highway 200 at the base of the National Bison Range (NBR). Image 1 displays the Stranahan research site in the floodplain.

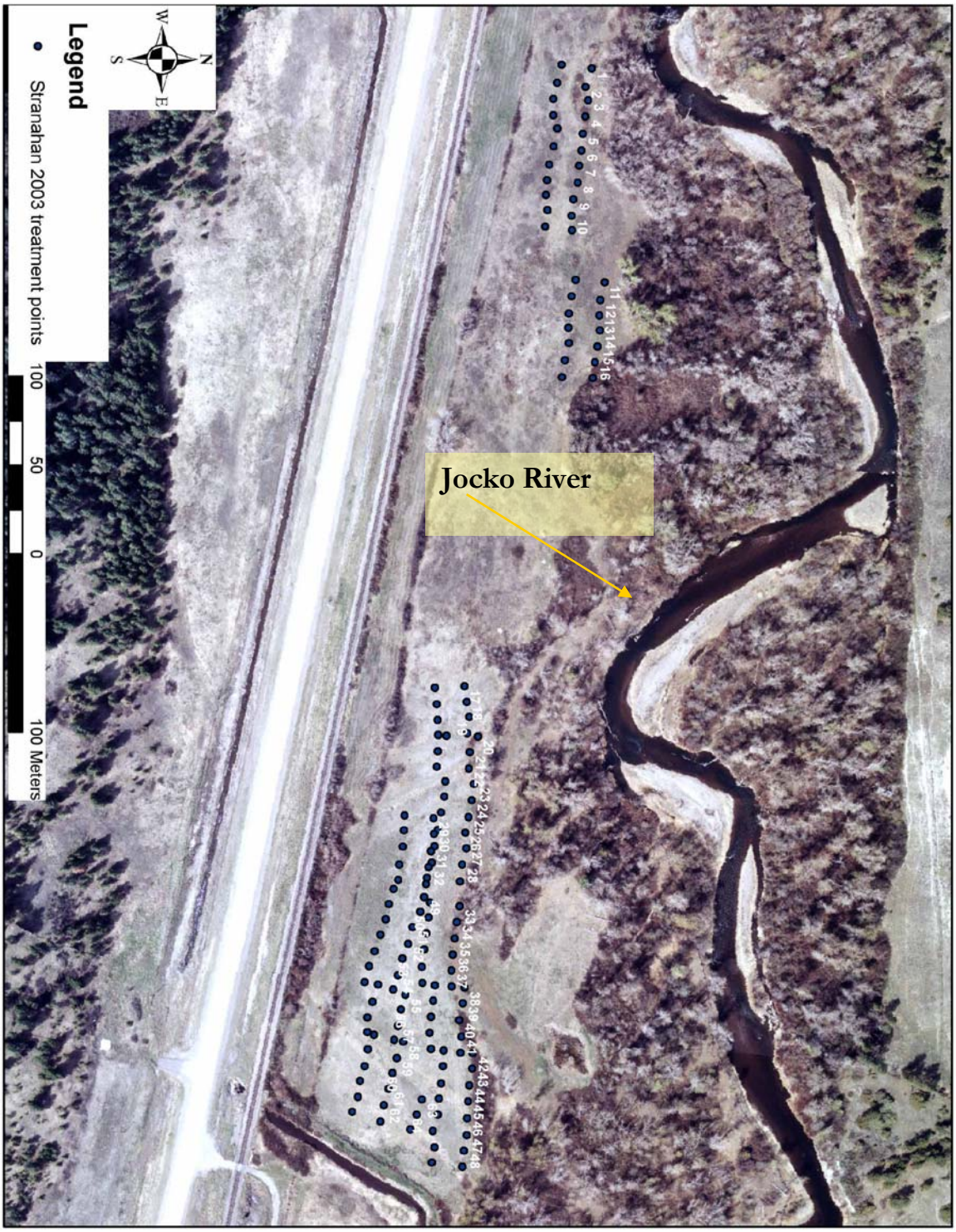

Image 1. Stranahan research site and replicated block design

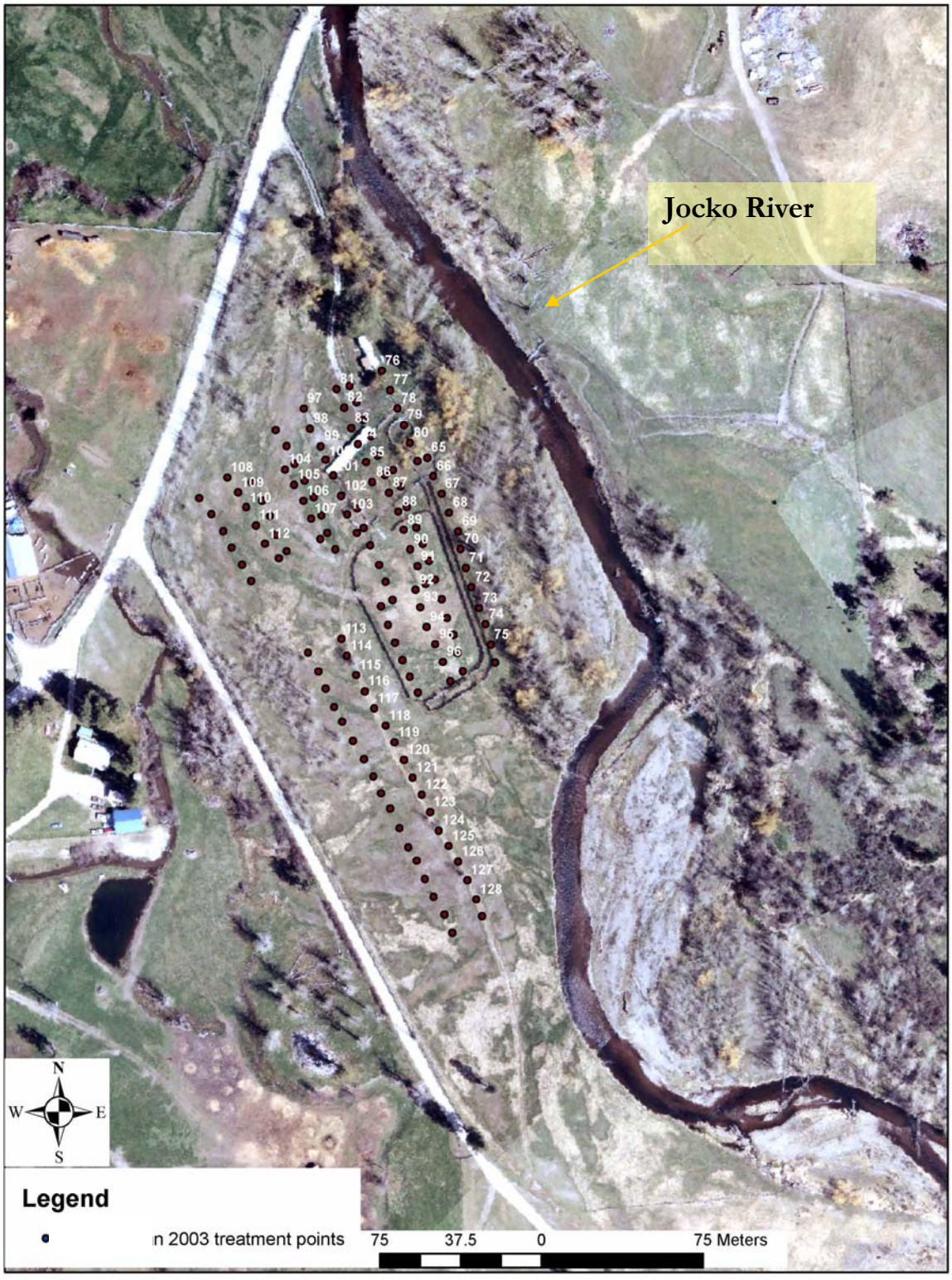

Image 2. Powell research site and replicated block design

Historically the Stranahan site was planted in crops and plowed annually, followed by planting in pasture grasses for haying. When CSKT purchased the Stranahan property, there were a variety of ruderal, weedy forbs interspersed with pasture grasses throughout the floodplain. After the Tribe purchased the property, they applied 2,4-D amine to reduce the nonnative forbs.

The Powell research site is at the northwest end of the Jocko Valley and borders the Jocko River between numerous private ranches. Image 2 displays the Powell research site. The Powell site was formerly managed as a cattle operation. A trailer house, barn, animals, corral, and various fenced pastures were removed from the site in the spring of 2003 in preparation of planting. The vegetation on this site included nonnative pasture grasses, the deep rooted *Phalaris arundinaceae* (Canary reed grass), *Centaurea maculosa* (Spotted knapweed), and *Hyoscamus niger* (Black henbane). The Powell site was plowed in May 2003, prior to planting and seeded in native grasses and forbs.

Table 1. Mean annual growing season temperature and precipitation (NWS 2005)

Weather Station - St. Ignatius, Montana Elevation 883.9 m (2900') 47°19'N/114°06W (NWS)		
Month	Mean Precip. Inches (cm)	Mean Temp. F (C)
Annual	**16.54 (42.0)**	
March	1.16 (2.9)	38.8 (3.8)
April	1.42 (3.6)	46.6 (8.1)
May	2.59 (6.6)	54.1 (12.3)
June	2.30 (5.8)	61.2 (16.2)
July	1.39 (3.5)	67.0 (19.4)
August	1.28 (3.3)	66.9 (19.4)
September	1.36 (3.5)	57.2 (14.0)

In general, the Jocko Valley is dominated by cold winters and warm summers with most of the annual precipitation occurring April thru June. The closest meteorological station is St. Ignatius, Montana, with an average annual precipitation of 42 cm (Table 1). Table 1 includes a summary of the mean

annual and growing season precipitation and the mean monthly temperature for the growing season recorded at the National Weather Station, St. Ignatius, Montana, from July 1, 1948 to December 31, 2004.

The Stranahan and Powell sites are similar in soil classification, topography, and flooding frequency. Both sites show visual evidence of shallow underground springs meandering along the floodplain likening to changes in vegetation. The geographic coordinates of the sites and a general site characterization are provided in Table 2.

Table 2. Site location NRCS soil survey classifications

Site	Location	Habitat Type	NRCS Soil Survey Classification	Flooding Frequency	Elevation
Stranahan NRCS Soil Survey Ravalli Quad (USDA, 1998)	S31 T18N, R20W and R21W, Lake & Sanders County	*Pinus ponderosa/ Cornus stolonifera*	Lamoose (gravelly) loam on 0-2% slopes	Occasional Brief, Jan-June	975.4 m (3200')
Powell NRCS Soil Survey Saddle Mtn Quad (USDA, 1998)	S21 T17N, R20W, Lake County	not typed	Lamoose (gravelly) loam on 0-2% slopes	Occasional Brief, Jan-June	994.8 m (3100')

Site Characteristics

Precipitation

Figure 1 displays the monthly mean precipitation in cm based on the St. Ignatius, Montana climatological data between 1948 and 2004 in addition to monthly precipitation in for the years 2002, 2003, and 2004. In 2002, the year prior to planting, the annual precipitation was 37.6 cm, 4.4 cm below the historical mean precipitation of 42.0 cm. Precipitation for 2002 was above the historical mean from May to July and fell below the mean for the remainder of 2002. In the planting year of 2003, the annual precipitation was even lower, at 31.0 cm, 9 cm below the historical mean. Shortly after planting in the end of May, 2003, precipitation fell well below the mean in June, July, and August, causing a drier than average summer. The annual precipitation in 2004 was 39.9 cm, only 2.1 cm below the historical mean.

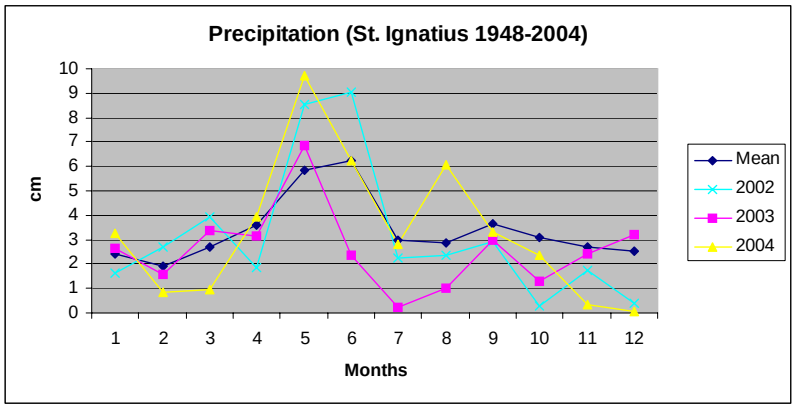

Figure 1. Mean precipitation and annual precipitation for 2002 - 2004

Soil

In the spring of 2003, prior to planting, soil samples were collected to a shallow rooting depth of 9" with a soil auger to characterize the soil of the two sites. From these samples the following was measured: Soil texture, pH, sand, silt, and clay (SSC) content, PO_4, C and N.

Table 3 provides the mean and standard error of the soil sample results for SSC, pH, SWC, PO_4, C, and N.

Texture (Sand/Silt/Clay) The soil samples collected at both research sites fall in the loam and sandy loam soil classes on the USDA textural triangle (Pierzynski et al. 2000) with a greater percentage of sandy loam soil at the Powell research site and a greater percentage of loam soil at the Stranahan research site. Figure 2 shows the percentages of sand, silt, and clay in each of the composite samples.

Water Holding Capacity (WHC). The percent water holding capacity of the soil samples for the Stranahan site varied between 14% and 21%, while the Powell samples range between 18% and 24%. The soil water content and percent clay show a weakly positive linear relationship on Stranahan and Powell research sites (R^2 = 0.78, 0.64, respectively). Figure 2 and Figure 3 show the relationship of percentage of clay in the soil and the soil water content.

pH. The pH ranges between 5.8 and 6.2 on the Stranahan site and between 5.8 and 7.0 on the Powell site. These ranges are slightly acidic to neutral. Most of the samples are within the normal range for nutrient availability. pH values below 5.6 or above 6.8 can cause nutrient deficiencies (Evanylo et al. 2000).

Phosphorus Extraction (*ug* PO^4/g). The Bray 1 soil test was used for phosphorus extraction (Kurtz 1987). Phosphorus as phosphate (*ug* PO^4/g) in Bray 1 soil extractions are higher on the Powell site and range between 25.6 and 93.7 *ug* PO^4/g. The Stranahan site phosphate ranges between 2.3 and 12 *ug* PO^4/g. For plant growth, the optimum value for the Bray-1 test is 30 mg P/kg (Pierzynski et al. 2000). The mean value for phosphate on the Stranahan soils, 7 mg

P/kg, falls below the optimum value, and the mean value on the Powell site, 56 mg P/kg is greater than the optimum value.

Total Carbon (C) and Total Nitrogen (N). Total percent C and N in soil samples are higher on the Powell Site (C 4.17% to 7.20% and N 0.38% to 0.66%) with an average C:N ratio at 11:1. The lower percent C and N at the Stranahan site (C 2.27% to 3.58% and N 0.20% to 0.29%) average a C:N ratio at 12:1.

Table 3. Mean and SE for Soil test results for composite samples (SSC, pH, SWC, PO_4 ,C and N)

	Soil Sample Results								
	% sand	% silt	% clay	pH	% SWC	*ug* PO4/g soil	%N	%C	C:N
	Stranahan Research Site								
Mean	42.50	36.75	20.75	6.0	0.23	7.07	0.23	2.75	12:1
SE	2.85	1.46	1.6	0.06	0.01	1.37	0.01	0.17	0.18
	Powell Research Site								
Mean	58.8	30.25	11	6.5	0.25	56.38	0.51	5.53	10.8:1
SE	2.95	2.05	1.13	0.13	0.01	7.63	0.03	0.31	0.09

See Appendix 2 for a discussion of Stranahan and Powell soil results and Appendix 1 for a discussion of plant-fungus mycorrhizae and nutrient acquisition.

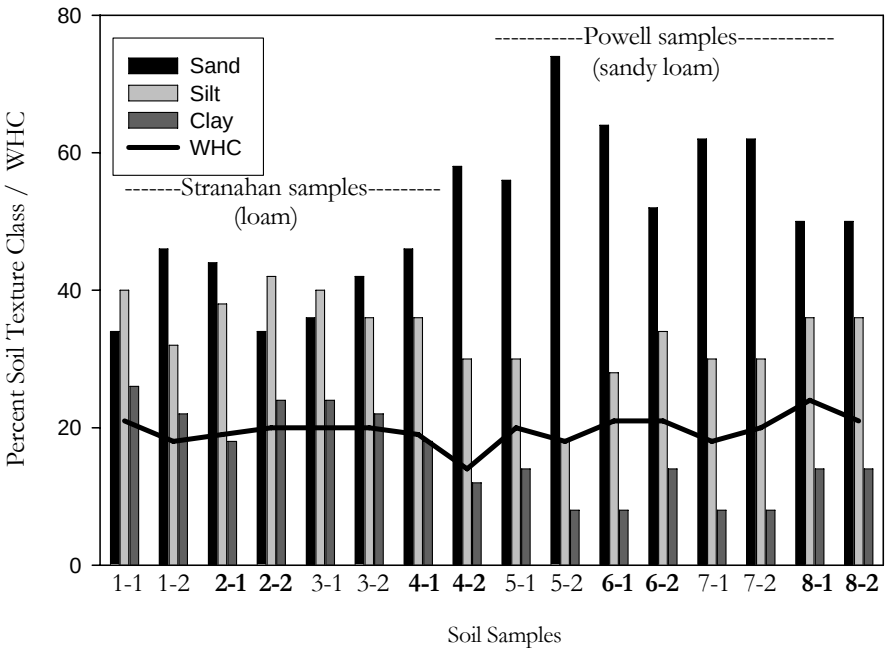

Figure 2. Soil texture and soil water content

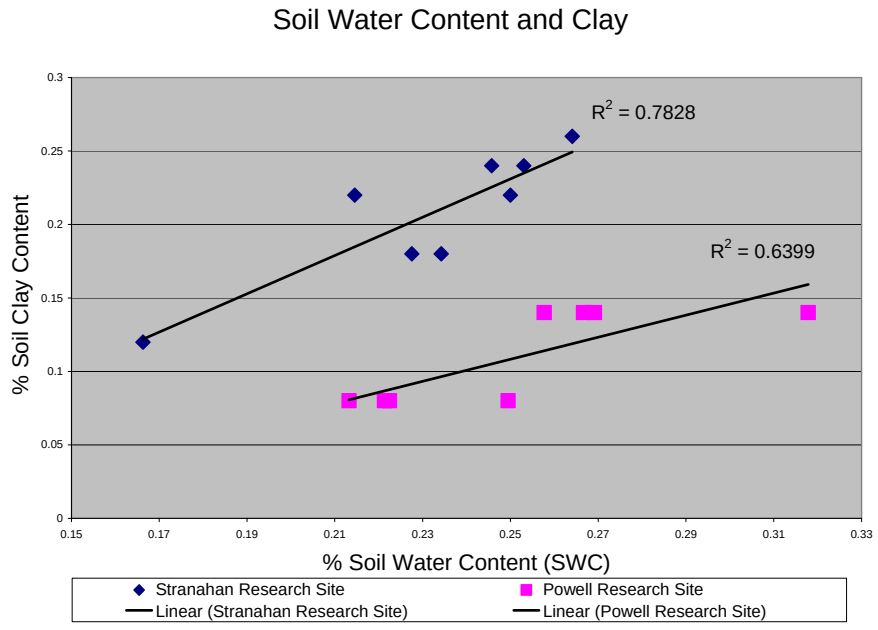

Figure 3. Clay content and soil water content

STUDY DESIGN

A field study and a greenhouse study were designed to determine the survival and biomass of *Populus tremuloides* (Aspen)*, Populus trichocarpa* (Black cottonwood), *Salix bebbiana* (Bebb's willow)*,* and *Cornus stolonifera* (Red osier dogwood) when inoculated with mycorrhizal fungal inoculum. The field study was a randomized block design with four replications on two separate floodplain sites. There were 16 plots in each randomized block (4 species x 4 treatments). The four treatments included two commercial inoculum products, Mycopak and Biogrow, forest soil inoculum, and a control.

The commercial inoculum products were a mix of AM and EM spores or propagules. Table 4 includes a summary of commercial inoculum products used, species, the form, number of fungal species, and spores by weight.

Table 4. Summary of commercial mycorrhizal inoculum treatments.

Inoculum	Form	AM Spores & Species	EM Spores & Species
Endo Ecto Mycopak Reforestation Technologies International	Pak	350/Pak *Glomus intraradicies*	800,000/Pak *Pisolithus tinctorius* *Rhizopogon* (4 spec) *Scleroderma*
Biogrow Endo/Ecto Mycorrhizal Applications, Inc.	Granular	60,000/lb *Glomus intraradicies, G. mosseae, G. clarum, G. monosporus, G. deserticola, G. brasilianum, Gigasport margarita*	110,000,000/lb *Pisolithus tinctorius* *Rhizopogon* (4 spec)

The native forest soil treatment was collected fresh each day from near the planting sites and kept cool. Perry and Amaranthus (1990) suggest collecting soil from a host plant in the planting vicinity of the same species and from an immature specimen. Little soil is needed,

approximately one-half cup per seedling at the time of planting with no particular attention to placement. They suggest that the time between collecting the soil and adding it to the planting hole should be minimized. The soil should not be allowed to dry out. Fresh forest soil inoculum is viable for a couple of days compared to years for dried propagules. The soil for this research project was collected 6-12 inches (15.24 – 30.48 cm) below the soil surface from an immature host plant and near the fine roots of each of the four species to increase the chance of collecting pieces of root, and fungal hyphae, and spores. Fresh soil was collected each day of planting. Soil was collected from below *Populus tremuloides, Populus trichocarpa,* and *Cornus stolonifera* on the edge of the Stranahan and Powell sites. Given neither research site contained *Salix bebbiana*, soil was collected from approximately 3 miles downstream of Powell on the Jocko River and applied at both planting sites. Treatments were added at the time of planting by using one Mycopak, one tablespoon of Biogrow, or approximately one-half cup (one palm full) of forest soil.

Seedling stock included 10 cubic inch seedlings in conetainers (root depth 8" [21.5 cm]) grown by Bitterroot Restoration, Inc. (BRI) of Corvallis, Montana. Seed source recorded by BRI for seedlings were: *Populus tremuloides* and *Populus trichocarpa* Deer Lodge; *Salix bebbiana* and *Cornus stolonifera*, Ravalli County. The seedling shoots were approximately 10 inches in height. The seedlings were dormant at the beginning of planting and were no longer dormant by the end of planting. Seedlings were watered prior to planting and clipped to a height of seven inches to reduce leaf area and risk of desiccation. Following planting, 2' x 2' brush blankets were installed to reduce neighboring plant competition for resources. In addition, 18" VexPro tree protectors were installed on each seedling to reduce animal browse.

All plots were labeled and color coded in the north corner for tree planters to reduce the risk of error in planting. With the assistance of a CSKT Forestry tree planting team, the seedlings and treatments were installed beginning in May of 2003, with 50 seedlings planted per plot. Following planting, information from Bitterroot Restoration, Inc. indicated *Populus trichocarpa* and *Salix bebbiana* had been treated with a watered-in mycorrhizal inoculum at the nursery. Five seedlings of each species were sampled to verify absence or presence of AM or EM structures in the roots.

In both 2003 and 2004, the height of each seedling was measured in centimeters, ocular estimates of biomass in grams were made based on reference samples, and vigor ratings were assigned from 1 to 3 with 3 having the greatest vigor at the end of August. Further, for each year two seedlings were collected from each plot and composited for a measure of the percent AM inoculation and presence of EM in the root.

Seedlings were planted in the CSKT greenhouse mimicking the field planting treatments to evaluate the study design in a controlled environment. Ten seedlings of each species were planted from the same BRI lot numbers as the field plantings for each treatment.

METHODS

Data Collection

Field Plantings

Biomass. Aboveground biomass of each seedling was estimated using a reference estimate method modified from Bonham (1989). In 2003, to create reference samples, 10 seedlings of *Populus tremuloides* (Aspen), *Populus trichocarpa* (Black cottonwood), *Salix bebbiana* (Bebb's willow), and *Cornus stolonifera* (Red osier dogwood) were collected from outside the plots. Seedling shoots were photographed then dried at 60°C. Shoots and roots were weighed after 1, 2, 3, and 4 days to verify final dry weight of biomass in grams. The photos and biomass of shoots were used to estimate aboveground biomass of all seedlings in 2003 and 2004. After estimates of biomass were made in 2003, two random samples were collected from each plot for root staining to and verify AM and EM fungal inoculation, discussed below. In an attempt to increase precision in biomass estimates for 2004, the shoot samples collected were photographed, dried, and weighed in grams to provide additional reference samples for estimating biomass.

Survival and Vigor. For survival, the number of living seedlings planted in each of 128 plots were counted at the end of 2003 and 2004 growing seasons. Because of dormant season plantings some plants were planted that were likely dead as no growth was observed. These were not included in survival ratings. Between 47 and 51 live seedlings were planted per plot and percent survival was calculated by the total seedlings surviving at peak standing crop by

the number planted alive, then multiplied by 100. The same process was followed for 2004, taking into account the seedlings sampled for inoculation.

AM and EM Infection. For a measure of AM and EM inoculation, using a random number calculator, two seedlings were collected from each plot during peak standing crop in 2003 and 2004 and dried. Several fine-root pieces were collected from each of the two roots collected to create a composite sample. Roots were cleared the roots in 10% potassium hydroxide (KOH), acidified in 1% hydrochloric acid (HCL) solution, and stained with Trypan Blue. Roots were stored in lactoglycerol until mounted on slides. Using a "squish preparation," two slides were prepared for each composite sample using five fine root pieces per slide.

At 200x magnification, a total of 100 stops per composite sample (50 stops per slide) were scored for presence of AM and EM fungal structures. For AM inoculation, vesicles, arbuscles and hyphae were scored but only AM hyphae was used as a general measurement of percent AM colonization. Presence of septate hyphae and the fungal mantle were used to identify presence of EM fungus in the root. A different calculation is used to determine percent colonization of EM fungi. Therefore EM structures were only used as a measure of incidence (Chilvers 1987). A note to the reader: There are other types of mycorrhizal associations other than AM and EM that have a septate hyphae and fungal sheath/mantle, including ectendo, arbutoid and monotropoid associations which are similar to EM, but have specialized anatomical features (Brundrett 1996). No distinction was made between the different associations.

Greenhouse Plantings

On April 9, 2004, seedlings were planted in the CSKT greenhouse mimicking the field planting treatments to evaluate the study design in a controlled environment. Ten seedlings were used from the same BRI lot numbers as the field plantings for each treatment. Commercial sterile soil was used as the planting medium. On October 5, 2004, 180 days after planting, the height of seedlings were measured and the shoots and roots were dried at 60° C for seven days. Thereafter shoots were weighed in grams for biomass, and roots were sampled to measure percent AM hyphal infection and presence of EM hyphae (septate) in the roots using the method described above.

Data Analysis

Field Plantings

All seedlings planted in the four replications per site (128 plots) were subject to measurement and analysis by species as a population. Random samples of seedlings collected from each plot were analyzed as samples of the population. Each of the four shrub/tree species selected were analyzed separately because each species have different physiological and morphological growth habits (Bonham 1989) and may react differently to mycorrhizal inoculation. Stained samples of BRI stock analyzed for pre-inoculation are displayed in a table.

Ocular Estimates of Biomass as Reference Samples. In 2003, ocular biomass estimates were made from reference samples. In 2004, ocular biomass estimates were made from the initial reference samples, as well as reference samples from the 2003 random seedling samples. Paired observations of the 2003 ocular estimates of biomass and actual biomass weights of random samples were analyzed in SPSS for each species with simple linear regression using the actual biomass as the dependent variable and ocular estimates as the independent variable. A validation f-test (Draper and Smith 1981) was used to determine bias, if any, in the ocular estimates. The F-test for each species is displayed in Appendix 3 (Table 6). Pearson's simple correlation coefficient was calculated in SPSS and used to determine the strength of the linear association for each species between actual and estimates of biomass. Scatter plots were used to verify the linear relationship of actual versus estimated biomass, also displayed in Appendix 3.

Actual and Ocular Estimates of Biomass. Biomass values were transformed using the natural log because of unequal variances of biomass for all species. Following transformations, the data were analyzed in SPSS using a general linear model to evaluate: 1) whether the commercial mycorrhizal inoculum or forest soil had any affect on biomass or survival for each species compared to controls; and 2) whether forest soil inoculums provided significantly quantifiable mycorrhizal inoculation in comparison to commercial inoculums. Three explanatory variables were explored: Site, treatment, and species, and their interactions. Mean estimates of biomass for POPTRE and POPTRI are displayed in error bars comparing treatments on the Powell and Stranahan research sites for 2003 and 2004. The mean biomass for all species randomly sampled in 2003 and weighed in grams are displayed in error bars for each treatment on the Powell and Stranahan research sites.

Survival. Percent survival was transformed with arcsine and analyzed in SPSS for 2003 and 2004. ANOVA was used to evaluate whether species, mycorrhizal treatments, or site had an affect on survival in both years. Seedling survival (%) for each species are displayed in clustered bar charts for 2003 and 2004 comparing treatments to controls on Powell and Stranahan.

AM and EM Infection. Percent AM mycorrhizal hyphae was transformed with arcsine and analyzed in SPSS using a general linear model to evaluate three explanatory variables: Site, treatment, and species, and their interactions: Actual counts were used to evaluate whether these explanatory variables had an affect on incidence of AM hyphae or EM septate hyphae.

Greenhouse Plantings

Biomass was transformed with a natural log and percent AM hyphal infection was transformed with an arcsine. Biomass and percent AM hyphal infection were analyzed as dependent variables in SPSS with a general linear model to evaluate possible affects of the explanatory variables treatment and species. Bar charts are used to display presence of AM and EM fungal hyphae in the root and mean biomass is displayed in error bars for each species and treatment.

RESULTS

Results for the field study and greenhouse study are presented in separate sections. For the field study, estimated biomass results are displayed for *Populus tremuloides* (POPTRE) and *Populus trichocarpa* (POPTRI). Those species were found to have strong linear correlations between ocular estimates of biomass and actual biomass (r=0.87 and 0.93, respectively); whereas *Cornus stolonifera* (CORSTO) and *Salix bebbiana* (SALBEB) had weak associations of r=0.71 and r=0.50, respectively. Ocular estimates and actual biomass scatter plots and a discussion of the results are presented in Appendix 3. Results of actual biomass, survival, and mycorrhizal root colonization are displayed next. For the Greenhouse study, actual biomass and mycorrhizal root colonization are presented. Where biomass in grams are presented, one standard deviation is included and denoted by (±).

Table 5. Presence of AM and EM fungal hyphae per 100 microscope stops in BRI stock.

Sample	AM hyph	EM hyph
poptre BRI 1	0	0
poptre BRI 2	21	3
poptre BRI 3	2	2
poptre BRI 4	4	6
poptre BRI 5	0	4
salbeb BRI 1	17	23
salbeb BRI 2	1	3
salbeb BRI 3	7	16
salbeb BRI 4	1	1
salbeb BRI 5	0	12
poptri BRI 1	0	0
poptri BRI 2	0	0
poptri BRI 3	0	0
poptri BRI 4	0	22
poptri BRI 5	0	0
corsto BRI 1	0	0
corsto BRI 2	0	0
corsto BRI 3	0	0
corsto BRI 4	0	0
corsto BRI 5	0	0

Following planting in May, 2003, it was mentioned by BRI that some of the seedlings purchased may have been exposed to mycorrhizal inoculum in the nursery. Random samples of 5 plants of each species were tested for AM and/or EM hyphal presence (Table 5) and 4 out of 5 POPTRE had AM and/or EM hyphal presence, SALBEB had AM hyphae presence on 4 out of 5 plants and EM on 5 out of 5 plants. POPTRI showed EM presence on one seedling root and CORSTO showed no sign of AM or EM in the root.

The presence of hyphae in the root is not uncommon given that fungal spores can travel by air currents and other vectors (Allen 1993). POPTRE and SALBEB showed both AM and EM hyphal presence in the root. POPTRI showed EM presence on one seedling root and CORSTO showed no sign of AM or EM in the root. It was decided to continue with analysis of seedlings planted given the labor intensive installation, cost of seedlings for the project, and potential for exposure to fungal spores in a natural setting. In addition to inoculum exposure in the BRI greenhouse, seedlings may also have naturally been exposed to fungal spores prior to planting in the greenhouse and in the field prior to planting.

Field Results

Ocular Estimates of Biomass. Total estimated plant biomass of POPTRE and POPTRI was significantly affected by mycorrhizal inoculum treatments ($p<0.001$). In 2003, the mean estimated biomass for both species in all treatments was higher on the Powell site than the Stranahan site ($p<0.05$). On Stranahan POPTRE showed the greatest mean estimated biomass with forest soil (1.8 ± 1.2 g) and on Powell with Biogrow (2.4 ± 1.3 g). POPTRI showed the greatest mean estimated biomass on Stranahan and Powell with Biogrow (1.5 ± 1.3 g, 2.2 ± 1.2 g, respectively). Even though POPTRI showed the highest biomass on both sites with Biogrow, the mean biomass in this treatment group was greater on the Powell site (Figure 4). Regardless of species, all treatments showed greater influence on biomass estimates at Powell ($p=0.005$).

In 2004, total estimated plant biomass for POPTRE and POPTRI were again significantly affected by treatments ($p=0.001$). By the end of the second summer after planting, the highest estimated mean biomass for POPTRE and POPTRI varied between sites. For POPTRE,

forest soil inoculums (5.2 $\pm$ 2.8 g) again showed the greatest influence on the Stranahan site and Biogrow (5.25 $\pm$ 3.7 g) on the Powell Site. POPTRI had eye-catching growth in the control (10.7 $\pm$ 11.6 g) and forest soil (10.5 $\pm$ 9.2 g) treatments at Stranahan. Both species showed high estimates of biomass in one replication of plots at the Stranahan site in comparison to all other plots, regardless of site. On Powell, POPTRI again showed the greatest mean estimated biomass with Biogrow (4.1 $\pm$ 3.6 g). Figure 4 shows error bars for 2003 and 2004 for the mean biomass estimates on each site, species and treatments.

Research sites, in combination, show Biogrow had the greatest association with estimated mean biomass of POPTRE in 2003 and 2004 (2.1g, 4.9g), and POPTRI in 2003 (2.0g). The 2003 and 2004 means for ocular estimates of biomass are displayed in Appendix 2 for each 'species x treatment x site' ($P<0.05$) and in total for each species.

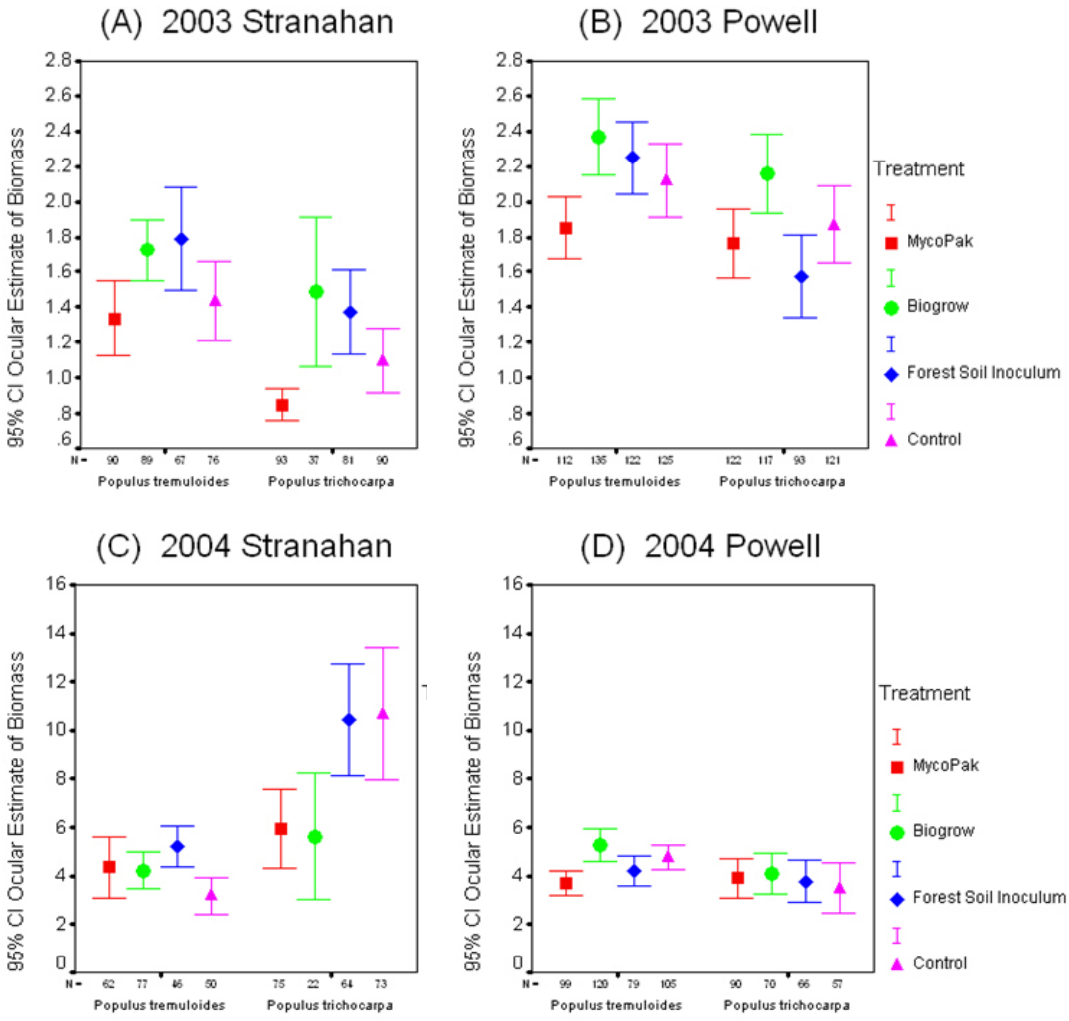

Figure 4. Ocular estimates of biomass for POPTRE and POPTRI on each site for 2003 and 2004.

Actual Biomass. Dry weight biomass for random samples collected for all species show biomass was significantly affected by treatments and the forest soil showed the greatest mean biomass for SALBEB (5.3 ± 6.1 g), POPTRI (5.9 ± 9.1 g), and CORSTO (10.3 ± 9.7 g) on Stranahan. POPTRE (6.0 ± 8.6 g) showed the greatest mean biomass with Mycopak ($p<0.05$). On the Powell site, all species had greater mean biomass with the Biogrow treatment:

POPTRE (3.4 ± 3.3 g), SALBEB (3.9 ± 3.1 g), POPTRI (3.1 ± 1.2 g), and CORSTO (4.9 ± 5.4 g).

Of the 339 seedlings sampled, for each species greater differences in mean biomass appear at each site between treatments and replications (p=0.007). On the Stranahan site Replication 1 showed a greater mean biomass in all treatment groups. See the error bar graphs in Figure 5 for mean biomass of samples collected from at the Stranahan and Powell sites.

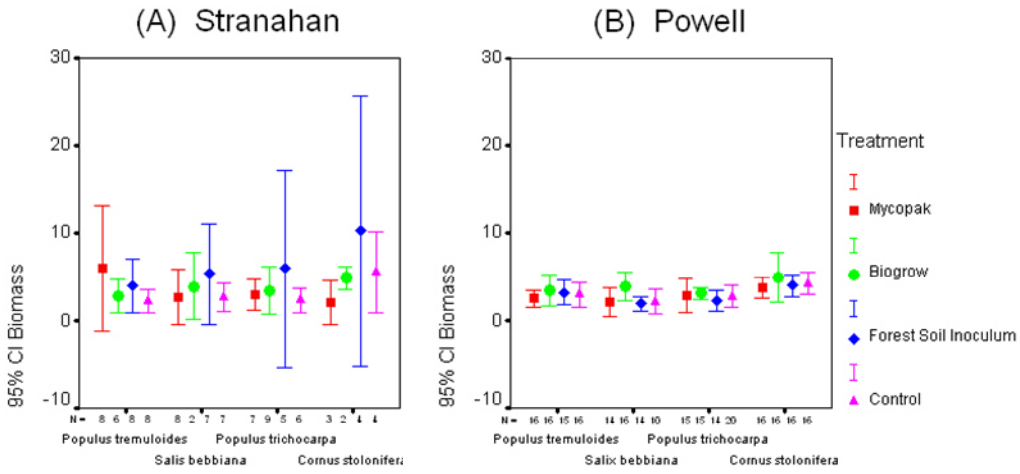

Figure 5. Mean biomass in grams from random samples collected at Stranahan and Powell

There are distinct site differences in seedling biomass between species with a greater span in error at the Stranahan site in the limited number of samples collected (94) versus the Powell site samples (245).

Survival. In 2003, overall seedling survival was 43.3%. By 2004 seedling survival decreased to 32.8% from initial planting; however, between 2003 and 2004 survival was 79.8% (Figure 6). In 2003, there were significantly more seedlings surviving (61%) on the Powell site (p<0.001)

compared to the Stranahan site. Of surviving seedlings, there were significantly more in the Biogrow treatment (30%) ($p<0.05$) and more POPTRE (29%) than other species ($p<0.05$). The treatment associated with the greatest POPTRE survival was Biogrow. In 2004, there were more surviving seedlings on the Powell site (45%). Of this number, there were significantly more POPTRE (33%) ($p<0.001$).

Although not significant in 2004, there were a greater number of surviving seedlings in the forest soil treatment for SALBEB and CORSTO on the Stranahan site in both years.

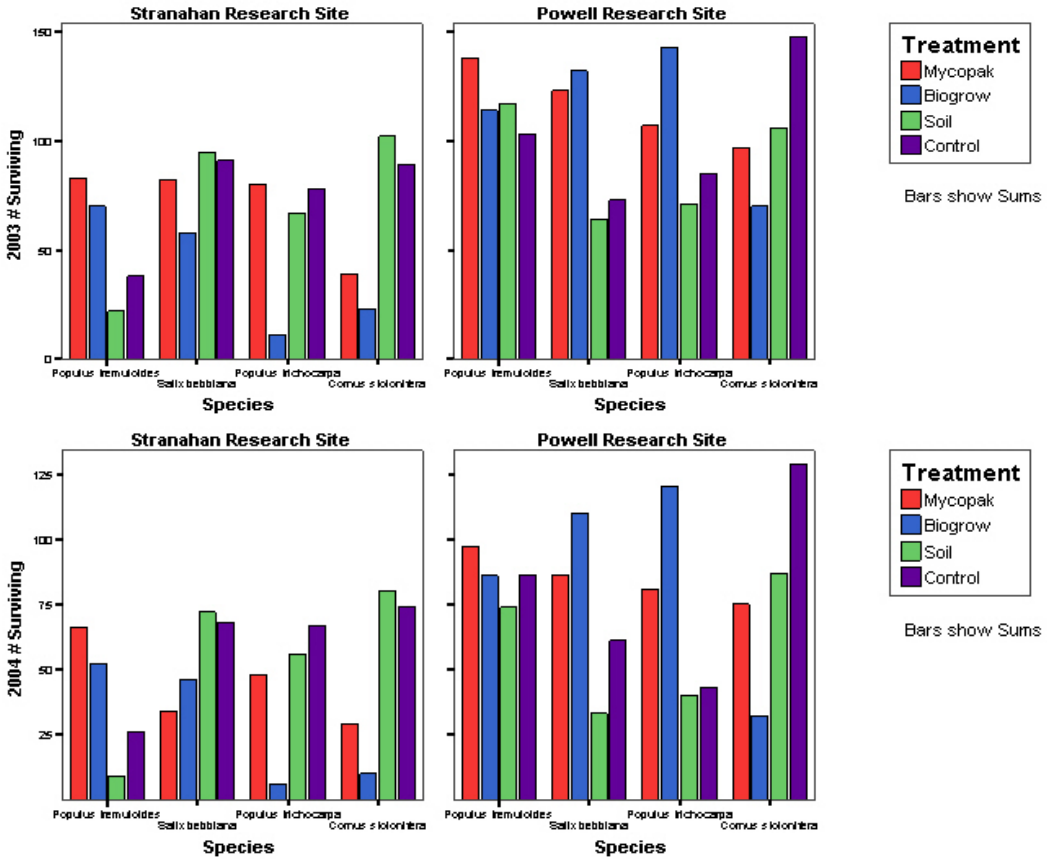

Figure 6. Seedling survival for 2003 and 2004 on Stranahan and Powell sites

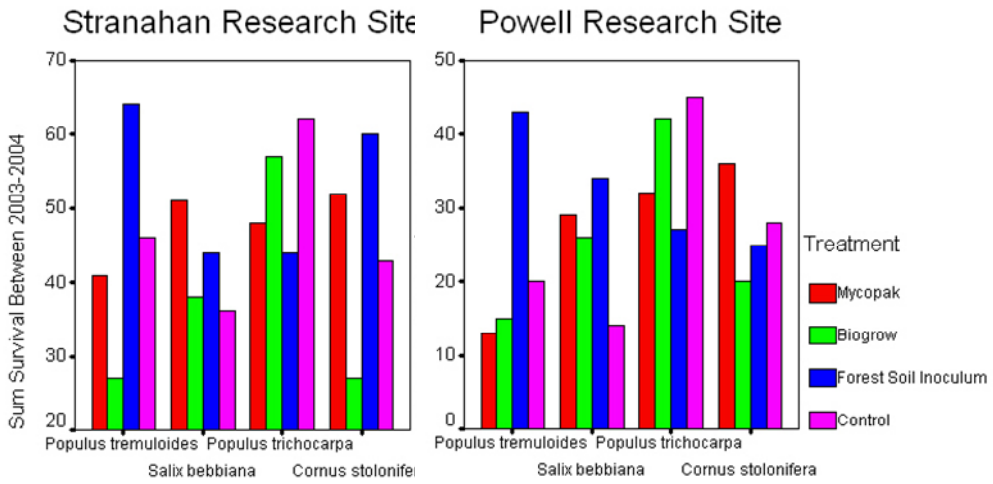

Figure 7. Survival between 2003 and 2004

Between 2003 and 2004 there were a greater number of POPTRE seedlings surviving in the forest soil treatment on both sites and for SALBEB on the Powell research site (Figure 7).

AM Percent Infection. In 2003, CORSTO had a significantly greater mean percent AM (30.2%) inoculation on the Powell site while POPTRI showed a significantly greater mean percent AM (14%) inoculation on the Stranahan site ($p=0.01$). In 2004, CORSTO showed a significantly higher mean percent AM inoculation on Stranahan and Powell (60.1% and 54.5%, respectively) than other species.

AM Versus EM Root Colonization. In 2003, the incidence of AM fungi versus EM fungi in the root was higher in all species and treatments. In 2004, incidence of EM fungi was higher than AM fungi in POPTRE with forest soil inoculum by 41%. In SALBEB, incidence of EM was higher than AM in the Mycopak treatment by 22% and with forest soil inoculum by 15%.

From 2003 to 2004, the percent increase of EM hyphae was higher than the percent increase of AM hyphae for three species: POPTRE, SALBEB, and POPTRI. A note to the reader: There are other types of mycorrhizal associations other than AM and EM that have a septate hyphae and fungal sheath/mantle, including ectendo, arbutoid and monotropoid associations which are similar to EM, but have specialized anatomical features (Brundrett 1996). A distinction was not made in septate hyphae, mantle, nor fungal species.

Greenhouse Biomass. The greatest mean biomass (g) for each species differed by treatment ($p<0.001$): POPTRE: Control 1.9g, Biogrow 1.8g; SALBEB: Forest soil 5.8g; POPTRI: Biogrow 9.8g and forest soil 9.7g; and CORSTO, Mycopak 9.0g (Figure 8).

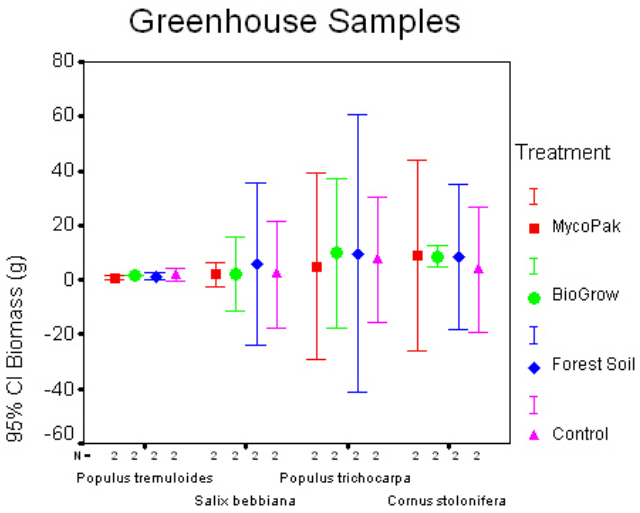

Figure 8. Greenhouse biomass samples

Greenhouse Root Colonization. The greatest mean percent infection of AM Hyphae varied between each species and treatment ($P=0.006$): POPTRE and CORSTO with Mycopak (3% and 17.9%, respectively) , SALBEB with Biogrow (25.5%), and POPTRI with forest soil (7.5%).

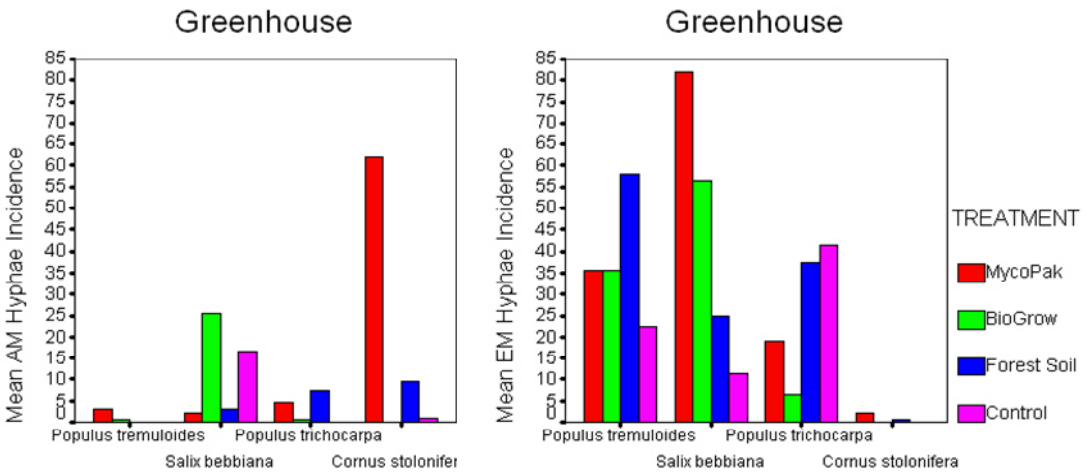

Figure 9. (A) Mean presence of AM hyphae and (B) Mean presence of EM hyphae for greenhouse samples.

Incidence of EM hyphae was greater across nearly all species and treatments compared to AM Hyphae (Figure 8). Incidence of AM hyphae was greater than EM hyphae three CORSTO treatments. POPTRI and CORSTO biomass correlated to the greatest incidence of AM and EM hyphae in the root.

Out of 200 stops for each 'species x treatment' POPTRE had the greatest mean incidence of EM fungal hyphae in its root in the forest soil (58) and the highest incidence of AM in the Mycopak treatment (3). SALBEB had the greatest incidence of EM in Mycopak (82) and AM in Biogrow (25). POPTRI had the greatest mean incidence of AM (8) in the forest soil treatment and EM (38) in forest soil and (42) in control. CORSTO had the greatest mean incidence of AM (62) and EM (2) in the Mycopak treatment.

DISCUSSION

The Stranahan and Powell research sites are both Jocko River floodplains formerly managed for agricultural purposes. Both sites historically support a variety of flood plain species including the species selected for this project. To rehabilitate the floodplains, in 2003, 6327 seedlings were planted between the Stranahan and Powell research sites. Planting started in May of 2003 after a drier than normal 2002 fall. Precipitation for May, 2003 was higher than the mean historical precipitation, but well-below the mean for June through November (Figure 1), a crucial time for seedling survival. In 2003, 3590 seedlings died. Precipitation was higher in 2004 and fewer seedlings were lost in the second year. Seedling death, at least in part, may be attributable to the dry site conditions.

I hypothesized that seedling survival would be higher for all species in Mycopak, Biogrow, or native forest soil over controls. In 2003 POPTRE, SALBEB, POPTRI, and CORSTO (Powell only) had significantly higher survival in a treatment other than control. The exception for my hypothesis was CORSTO on the Stranahan site, which showed a greater number of seedlings surviving in the control group. In 2003, treatment significantly influenced survival, but in 2004, treatment was no longer significant. The higher number surviving in the control group for the Stranahan site may have been due to loss of complete plots of CORSTO. Of the 16 plots of CORSTO planted on Stranahan, six of those were lost in their entirety in 2003 and two more were lost in 2004. The random manner of planting placed many dogwood plots in drier areas. The dogwoods did not tolerate the extremely dry, cobbled conditions.

Treatment may have lost significance in 2004 survival because of overlapping AM and EM species. Over time, these overlapping species may have begun to show similar affects. Additionally, given the differences in sites, Powell had significantly more seedlings surviving than Stranahan. The difference in sites may be due to moisture. The Powell site soils may have had greater water holding capacity given the higher organic matter content in the soils. The Powell site also showed greater signs of accessible moisture in the areas with *Phalaris arundinaceae*, a grass typically growing in wetter areas. The height and shading ability of *Phalaris arundinaceae* may have helped increase seedling survival. Transpiration and shading under the *Phalaris* canopy kept seedlings leafy and green.

I further hypothesized that seedling biomass would be higher for all species in Mycopak, Biogrow, or native forest soil over controls, and the mean seedling biomass of each species in the forest soil treatment would be higher than other treatments. Results varied among species and treatments in the actual biomass of random samples removed from plots. My hypothesis held true on the Stranahan site where the mean biomass was significantly highest in the forest soil treatment for SALBEB, POPTRI, and CORSTO; Mycopak showed the highest mean biomass in POPTRE. On the Powell site, my hypothesis was correct in part. While forest soil inoculums were not associated with the greatest mean biomass, Biogrow significantly increased seedling biomass in all species.

The ocular estimates of biomass in the field did not correlate to actual biomass for CORSTO and SALBEB. The ocular estimates for CORSTO and SALBEB were not included in the above results. Making ocular estimates adapted from Bonham, 1989, looking at the height and reference samples did not work as well for CORSTO and SALBEB as the method worked for

POPTRI and POPTRE given the growth and branching habits. CORSTO and SALBEB are shrubbier species with extensive branching. The tree protectors used in the field also presented some difficulty in estimating biomass for CORSTO and SALBEB. In some cases these shrubby species were highly branched and packed tightly inside of the protectors.

In comparing the mean estimates of biomass, POPTRE showed the greatest mean biomass in 2003 and 2004 with forest soil (1.79 g, 5.23 g) on Stranahan and with Biogrow (2.37 g, 5.26 g) on Powell. Given that inoculum was collected from near the planting site, inoculum for POPTRE was collected in different locations for each planting site. This, and site characteristics, may account for the differences in estimated mean biomass between sites. This would suggest that forest soil collected near the Stranahan site may have had a higher inoculum potential than forest soil collected near the Powell site. Differences in seedling biomass may also be attributed to other differences in the forest soil, including possible introduction of saprobes, pathogens, and nutrient differences.

In both years, POPTRI had the greatest mean estimated biomass with Biogrow (2.16 g, 4.08g) on Powell, but only in 2003 on Stranahan (Biogrow 1.49 g). In 2004, the POPTRI control group showed a mean biomass of 10.67 g. This mean estimated biomass is considerably high given the growth of all POPTRI plots in Replication 1 on the Stranahan site, where all POPTRI seedlings showed greater growth than all other POPTRI plots. This growth may have been due to greater soil moisture in the location where that replication of plots was planted. Soil moisture and site differences had a great impact on growth and biomass.

The estimates of biomass for both species were higher on the Powell site in 2003 and higher on the Stranahan site in 2004. Likewise, the mean percent of AM hyphae in the root was

greater for most 'species x treatment' on the Powell site in 2003, and greater on the Stranahan site in 2004. Given the level of soil nutrients on the Powell site and the soil water holding capacity, in 2003 the seedlings may have received an early flush of growth from the moisture and soil nutrients, which typically increases the vegetative growth and biomass of plants.

Succession and fungal competition may also have influenced biomass and survival on both sites. Some species of EM fungi do not enter the root as quickly as AM fungal species (Allen 1993). Chen et al (2000) showed that AM fungi are quicker to colonize roots than EM fungi in some *Eucalyptus* seedlings. This successional transition in the *Eucalyptus* seedlings, from a greater proportion of AM fungi in the root to a greater proportion of EM fungi in the root are not due to decline in AM fungi, but rather the substantial increase of EM fungi within the root. Chen et al. (2000) noted when both fungi types were in the growth pot, EM replaced AM as the dominant mycorrhiza type in the *Eucalyptus* roots after several months of growth. While EM fungi generally produce a high growth rate response in *Eucalyptus*, results from one set of experiments cannot necessarily be used to produce the response of a different set of interacting plant and fungal symbionts (Lodge 2000).

The succession from AM to EM found through prior research (Chen et al. 2000, Lapeyrie and Chilvers 1985) in *Eucalyptus* spp. appears to be supported in field findings. Findings for presence of EM fungal septate hyphae were made based on the septate hyphae and fungal mantle on the root exterior. These structures also appear in other fungal varieties but species and associations were not differentiated in this experiment.

In 2003, all species showed greater incidence of AM fungal hyphae in the root. In 2004, AM incidence was still higher than EM incidence on most roots sampled; however EM fungal

hyphae showed a greater rate of increase in hyphal incidence in POPTRE, SALBEB, and POPTRI roots sampled. Because greenhouse seedlings were examined one time at 180 days, AM and EM incidence over time was not examined for the greenhouse trial. In the greenhouse seedlings, the incidence of EM hyphae in the roots were greater in POPTRE, SALBEB, and POPTRI in nearly all treatments. CORSTO, the only species not showing prior inoculation, treatments all contained greater incidence of AM fungal hyphae in the root. This finding is similar to the field samples where AM inoculation of CORSTO was greater than EM inoculation in the root, regardless of treatment, and the incidence of AM fungi was greater than the incidence of EM fungi from 2003 to 2004.

Brundrett et al. (1996) stated that POPTRE and SALBEB switch between an AM and EM relationship, and CORSTO and POPTRI typically maintain an AM relationship with symbiotic mycorrhizal fungi. My findings in the field and greenhouse seem to agree with Brundrett et al. (1996) for CORSTO in both the field (two summers) and greenhouse (180 days) findings. For all other species, in the controlled environment of the greenhouse, where seedlings were under no environmental stress, EM fungi may have a greater competitive advantage over AM fungi for inoculation sites early after inoculation treatment of the seedlings, reducing the ability of AM fungi to gain the early inoculation sites. In the field all other seedlings sampled showed greater incidence of AM fungi in the first year.

Prior inoculation of POPTRE, SALBEB, and POPTRI may have influenced outcomes of the above results. However, there were distinct differences between sites and interactions with the site and species/treatments that developed in the field and most likely were not due to the pre-inoculation, but were due to site conditions. Given POPTRE, SALBEB, and POPTRI

showed signs of EM hyphae and a fungal mantle on the root, there were fewer sites available for AM fungi to inoculate the roots. CORSTO roots, showing no signs of pre-inoculation, had more sites available for AM inoculation.

Results of a statistical analysis are only a piece of the puzzle when looking at the results of a field study. Observational and theoretical interpretations also need to be examined, as well as potential explorations for further study. With field studies, uncontrolled environmental differences must be examined in results. The moisture and nutrient differences on the Stranahan and Powell sites most likely had great influences over the seedlings planted. The pre-inoculation of seedlings by EM fungal hyphae in POPTRE and SALBEB were advanced in mycorrhizal succession at the time of planting and impacted the outcome of the greenhouse and field results. Mycorrhizal fungal species in the forest soil and on the floodplain were not identified prior to planting, reducing the ability to make seedling or mycorrhizae species-specific findings to relate back to statistical results.

Mycorrhizal fungi are generally thought to provide beneficial services to autotrophic plants; however, adverse impacts associated with both nonnative fungal symbionts and forest soil treatments may occur in forestry, restoration, horticultural, and environmental management practices (Schwartz et al. 2006). Commercial non-native mycorrhizal fungi added with the best of intentions in reforestation may lead to potential negative consequences. Recent literature explores invasive species problems associated with mycorrhizal inoculations. Further, impacts resulting from the addition of forest or field soil in an attempt to provide native mycorrhizal inoculum on site also may produce adverse impacts. Schwartz et al. (2006) recommends using local inoculum sources whenever possible; however, non-sterile cultures of inoculum can

result in the movement of saprobes and pathogens in addition to mutualists (Schwartz et al. 2006, Allen 1993).

Variable soil additions may also include nutrient additions of mineral soils that may impact seedling biomass and survival. One-half cup of top soil (mineral soil), approximately 200 grams, may contain 0.05 to 0.15% total N. Generally 1 to 3% of this organic N will mineralize annually to produce 15 to 70 kg N/ha/year (Pierzynski et al. 2000). Additions of field and forest soil, as well as planting sites, should be analyzed for nutrient content and mycorrhizal species to provide greater detail in research results.

CONCLUSIONS

Field experiments in contrast to laboratory and greenhouse studies tend to show greater variability given the number of conditions that cannot be controlled in nature. The results and discussion of this experiment did not take into consideration the many interactions in the soil profile nor the weedy, competitive species and conditions encountered above ground on the research sites. This research takes into account a narrower view of the direct correlation between the mycorrhizal treatments and mycorrhizal inoculation of AM and EM fungal symbionts where the potential for EM inoculation may not exist in a floodplain grassland. Specifically, this experiment was intended to look at the feasibility and use of forest soil in landscape restoration of native trees and shrubs. Control treatments where no fungal symbionts were added, showed lower survival than other treatments in most cases, but were not lacking in inoculation of AM and EM mycorrhizal fungi in the grassland floodplains.

As the study of mycorrhizae evolves to the landscape and ecosystem scale a greater number of studies will move from the lab to the field where a greater number of factors may be considered above and below the soil surface. During the time of analysis and completion of the within floodplain research, Schwartz, et al. (2006) identified several enlightening management considerations to follow when applying mycorrhizal inoculum in environmental management projects. In field studies a great standard of care should be exercised when utilizing mycorrhizal inoculum, including identification of mycorrhizal species on the planting site, host specific mycorrhizae needs, successional changes in mycorrhizal species, and a quality assessment of the true need for inoculum. Care should be taken in collecting and utilizing

native cultures to reduce saprobes and pathogens through sterilization techniques. Benefits to nontarget species should also be considered to minimize unintended negative impacts associated with invasive species and their ability to exploit soil resources to their own advantage. Increasing the growth of invasive species is counterproductive and costly to restoration practices.

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APPENDIX 1 – SOIL AND MYCORRHIZAE

Soil consists of minerals, organic matter, water, and soil organisms. Soil formation and quality (Evanylo and McGuinn 2000) is the combined effect of chemical, biological, and physical processes. Aboveground growth and biomass of plants depend largely upon the soil processes belowground that provide nutrients to plant communities (Pierzynski et al. 2000, Wardle 2002). Mycorrhizal fungi, part of the soil organic matter, help the plant acquire soil nutrients and water in exchange for photosynthetic carbon products (Harley 1971, Pierzynski et al. 2000, Wardle 2002). Allen (1993) defined mycorrhiza as "… a mutualistic symbiosis between plant and fungus localized in a root or root-like structure in which energy moves primarily from plant to fungus and inorganic resources move from fungus to plant." Mycorrhizal fungi are found in every terrestrial ecosystem and represent one of the largest biomass components of those ecosystems (Allen 1993). The fungal hyphae extend into the plant root, along the surface area of the root, and increase the plant's acquisition of nutrients beyond the depletion zone created by roots. (Allen 1993).

Two common types of mycorrhizal symbioses are endomycorrhizae (AM), where the hyphae penetrates the root cortical cell, and ectomycorrhizae (EM), where the hyphae do not penetrate the root cortical cell. These mycorrhizal fungi, heterotrophic organisms, are adapted to a mode of life where all their nutrient requirements are absorbed as soluble material from their substrates (Harley 1971).

Mycorrhizal hyphae length can range up to 50 meters or more per ml of soil, increasing its capacity to exploit a given volume of soil (Allen and Allen 1986) and increase soil aggregation (Evanylo and McGuinn 2000). Mycorrhizae increase nutrient transport to the host plant(s) (Allen 1993, Pearson and Jacobsen 1993, Chalot et al. 2002). For instance, smooth brome (*Bromus inermis*) inoculated with AM fungi *Glomus fasciculatum*, showed an increase in K, Ca and Mg (Chalot et al. 2002). Mycorrhizal inoculation increases water flow through its host and reduces resistance to water flow under water stress in the greenhouse (Bildusas et al. 1986). In addition, Allen (1982) found an increase in water movement in the host plant without water stress. Although plant species differ in the extent of their response to mycorrhiza, the symbiosis is typically linked to positive benefits to the host plant via increased rates of survival, growth, biomass production, and increased acquisition of water and nutrients (Allen 1993, Pearson and Jacobsen 1993).

Nitrate (NO_3-) and ammonium (NH_4+) are nitrogen forms utilized by plants. These forms of nitrogen are relatively mobile in the soil solution where there is potential for increased migration to roots for uptake (Ames 1983, Pierzynski et al. 2000) by the plant or transported by mycorrhizal fungi to the plant by ectomycorrhizae (Ames et al. 1983, Chalot et al. 2002) or endomycorrhizae (Ames et al. 1983, Martin et al. 1986). Both fungus and fine roots will immobilize substantial quantities of nitrogen in producing their own growth. Mycorrhizal hyphae, given its hyphal length and absorptive surface area, have the capacity to mine (spread and penetrate) substrate for nitrogen pools for and transport to the plant. Mycorrhizal fungi contain enzymes, used to break down organic nitrogen and alter forms of nitrogen in the soil for use by host plants (Allen 1993, Chalot et al. 2002).

Mycorrhizae are known to affect the inorganic phosphorus (P) nutrition of host plants. Phosphate is the major form of P available for plant uptake but is relatively insoluble in the soil solution (Allen 1993, Chalot et al. 2002, Yadav et al. 2006). Phosphorus obtained by AM fungus is translocated through the hyphae and taken up by the plant cell (Yadav et al. 2006). Pearson and Jacobsen (1993) define mycorrhizal efficiency as the ability of the fungus to increase photosynthesis and growth of the host by improving it's P supply. They found an increase in mycorrhizal efficiency by simultaneously labeling of the host plant with $^{14}CO_2$ and the external hyphae with ^{32}P (Pearson and Jacobsen 1993).

Various forms of organic matter in the soil increase the C content of the soil and improve the soil water holding capacity (Evanlyo et al. 2000). Soil microorganisms influence the availability of nutrients in the soil by decomposing soil organic matter and releasing or immobilizing plant nutrients to sustain and maintain healthy soils. In addition to their role in nutrient function, mycorrhizae play a key role in the process of soil aggregation of organic and mineral complexes at the levels of the plant community (net primary production and litter quality), individual root (rhizodeposition, soil water, root decomposition, soil aggregate penetration), and fungal mycelium (physical, biochemical, and biological mechanisms) (Rillig and Mummey 2006).

Healthy soils can be more specifically described as (1) sustaining biological activity, diversity, structure and productivity, (2) regulating and partitioning water flow, (3) filtering and degrading anthropogenic inputs, (4) storing and cycling nutrients, and (5) and supporting life aboveground. The assessment of soil quality may help explain biological activity in soils and provide land managers with a starting point for agricultural practices and management. The

assessment of soil quality requires quantification of critical soil attributes across high and low productivity areas to establish a range of values that are site specific (Evanylo and McGuinn 2000).

APPENDIX 2 – SOIL RESULTS DISCUSSION

There are distinct site differences between Stranahan and Powell in the amounts of C, N, and $P0_4$. In all samples, C, N, and $P0_4$ are in greater abundance on the Powell site. In the fall of 2002, prior to planting in 2003, livestock and an arena were removed from a portion of the Powell planting area (Image 2). Agricultural production was removed from the Stranahan site a year earlier than the Powell site. The decomposing manure remaining on the Powell site may have increased the organic matter and nutrients in the soil.

As much as 60% of the P in some animal manures is mineralized inorganic P. Thus, manured soils have higher levels of inorganic phosphorus (Pierzynski 2000). For plant growth, the optimum value for the Bray-1 test is 30 mg P/kg (Pierzynski et al. 2000). The mean value for phosphate on the Stranahan soils, 7 mg P/kg, falls below the reported optimum value, and the mean value on the Powell site, 56 mg P/kg is greater than the optimum value.

While total N in the soil is not completely usable by the plant, the ratio of C to N provides an insight to potential forms of inorganic N. A C:N ratio from 10:1 to 12:1 provide stable organic matter without a substantial increase (lower ratios) or decrease (ratios <30:1) of inorganic N, specifically plant available forms NH_4 and NO^3. Given the mean C:N ratios for Powell and Stranahan are 11:1 and 12:1, respectively, both study sites show a stable C:N ratio.

WHC is the ability of the soil to retain water (Evanylo et al. 2000). Soil fertility and its capacity to hold water directly influence the soil's ability to sustain plant life. The percent clay and organic matter in the soil of the research sites may increase both the soil's WHC and ability to

adsorb nutrients. Soil texture has been linked with the soils ability to hold water in its pore spaces. Clay soils tend to hold the most soil water, although with its matric potential of smaller pore spaces, not all of the water is available for plant use. In Figure 2 and Figure 3 the soil water content and percent clay show a moderately positive relationship for both sites. The moderate correlation between soil water content and soil texture is reflected more on the Stranahan site than the Powell site. Clay content on the Powell site is lower and the soil water content may have been related in part on the additional soil organic matter (Allen, 1993). The effect of organic carbon on the water held in soil is generally thought to be positive, but may not account for the types of carbon and the synergistic behavior with other soil properties (Krull et al. 2004).

Loamy soils provide the most plant available water where sand helps increase the pore space in soil and increases availability of water to plants (Allen 1993, Evanylo and McGuinn 2000). Both sites contain loamy soils. The clay content in loamy soils and soil organic matter help hold some of the nutrients in place, increasing access for green plants and mycorrhizal fungi. The sand content with a greater nitrogen and phosphorus level warrant concern in a floodplain. High nutrient levels in sandy floodplain soils increase nonpoint source nutrient loading. On the Powell site, where sand content is between 50% and 74%, nutrients may leach to ground water or runoff in to the Jocko River.

Soil pH influences nutrient solubility and availability to plants. The ability of clay soil and organic matter to sorb cations is higher with pH over 7, thereby increasing the soil's cation exchange capacity (CEC), or the soil's negative charge. Lower pH generally causes lower CEC because the higher concentration of H^+ ions in the solution neutralize the negative charges on

clays and organic matter. Therefore in soils with pH values below 7, there is a increasing chance of H^+ ions in the soil solution available for exchange (McCauley et al. 2003). With mean pH values of 6.2 (range 5.8 to 6.2) and 6.4 (range 6.3 to 7) on the Stranahan and Powell sites, the lower pH values may increase nutrients in the soil solution.

APPENDIX 3 – PAIRED SAMPLES OF BIOMASS

Pearson's correlation coefficient showed a strong linear association between the actual biomass and ocular estimates of biomass for POPTRE (r =.868) and POPTRI (r =.932). The linear association of actual and estimated biomass for SALBEB (r =.500) and CORSTO (r =.708) showed weak associations. See scatter plots of actual biomass and ocular estimates of biomass in Figure 10.

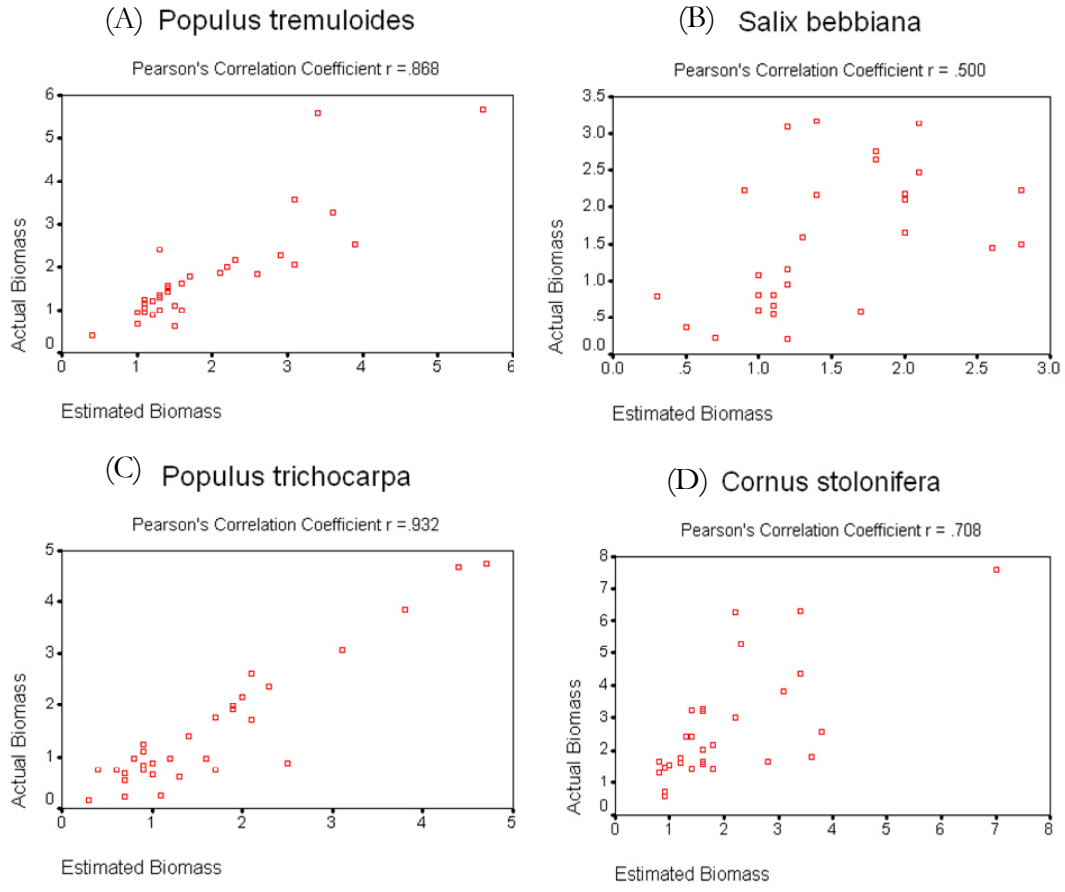

Figure 10. Scatter plots and Pearson's Correlation Coefficient of paired samples for (A) *Populus tremuloides*, (B) *Salix bebbiana*, (C) *Populus trichocarpa*, and (D) *Cornus stolonifera*.

The F-test for verification of nonbias in ocular estimates showed POPTRE ($F_{1,30,.05}$=4.17), SALBEB ($F_{1,26,.05}$=4.23), and POPTRI ($F_{1,30,.05}$=4.17), lack of significance, indicating the ocular estimates of biomass were unbiased. The F-test is significant for CORSTO ($F_{1,27,.05}$=4.21) and indicates the ocular estimates of biomass are biased. See Table 6 for a summary of F-tests for ocular estimates for biomass in the field.

Table 6. F-test for bias of ocular estimates of biomass. B_0 represents actual biomass and B_1 represents estimated biomass.

Species	df_1,df_2	B_0	B_1	F-calc	F(df_1,df_2,.05)
Populus tremuloides	1, 30	-0.049	0.972	0.949	4.17
Salix bebbiana	1, 26	0.463	0.729	1.359	4.23
Populus trichocarpa	1, 30	-0.185	1.022	3.724	4.17
Cornus stolonifera	1, 27	0.772	0.951	**8.307**	**4.21**

For CORSTO, my ocular estimates of biomass in the field were underestimated in 20 out of 29 seedlings when compared to the weighed samples of the same seedlings. The SALBEB and CORSTO seedlings were a bit more difficult to estimate in the field given their branching habits compared to POPTRE and POPTRI. See Images 4 through 7 for comparison of branching and growth of each species.

Because of the reduced correlation between ocular estimates of biomass and actual biomass for SALBEB and CORSTO, and bias in CORSTO samples, these two species were not used in the ANOVA for estimated biomass. Appendix 3 includes the mean estimated biomass of these species for each treatment and site.

Image 3. *Populus tremuloides* (Aspen)

Image 4. *Salix bebbiana* (Bebb's willow)

Image 5. *Populus trichocarpa* (Black cottonwood)

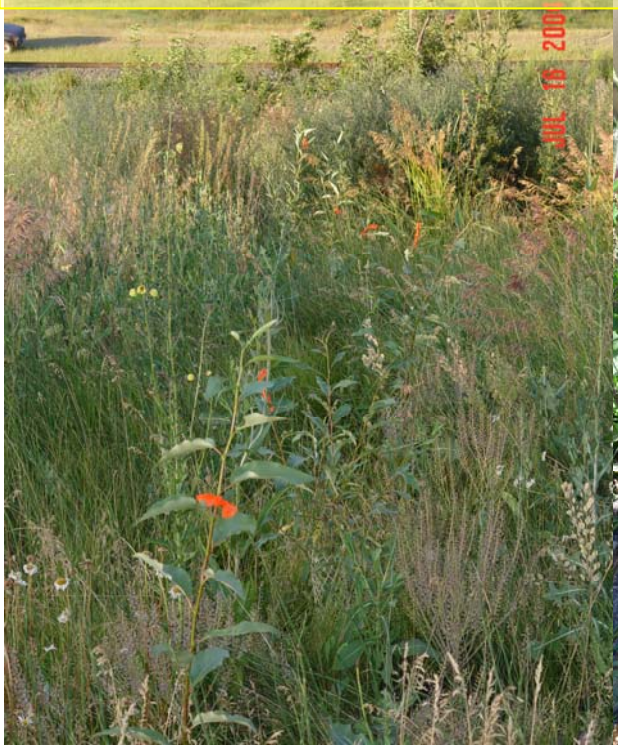

Image 6. *Cornus stolonifera* (Red osier dogwood)

Images 3 to 6. (3) *Populus tremuloides* (4) *Salix bebbiana* (5) *Populus trichocarpa* (6) *Cornus stolonifera*

APPENDIX 4 – ESTIMATES OF BIOMASS

Research Site	Species	Treatment	2003 Mean g	2004 Mean g
Stranahan	*Populus tremuloides*	Mycopak	1.3356	4.3548
		Biogrow	1.7236	4.2338
		Forest Soil Inoculum	1.7881	5.2348
		Control	1.4329	3.1840
	Salix bebbiana	Mycopak	.8840	4.5491
		Biogrow	1.3407	8.5133
		Forest Soil Inoculum	1.0279	5.3947
		Control	.7935	4.7323
	Populus trichocarpa	Mycopak	.8505	5.9267
		Biogrow	1.4892	5.6273
		Forest Soil Inoculum	1.3753	10.4625
		Control	1.0978	10.6767
	Cornus stolonifera	Mycopak	1.2600	4.6714
		Biogrow	.9583	3.6400
		Forest Soil Inoculum	1.1000	8.9480
		Control	1.6225	8.1204
Powell	*Populus tremuloides*	Mycopak	1.8491	3.6919
		Biogrow	2.3674	5.2575
		Forest Soil Inoculum	2.2484	4.2089
		Control	2.1216	4.7638
	Salix bebbiana	Mycopak	1.3333	3.0280
		Biogrow	2.2304	11.0380
		Forest Soil Inoculum	1.4719	3.9783
		Control	1.1488	3.7705
	Populus trichocarpa	Mycopak	1.7623	3.9044
		Biogrow	2.1581	4.0843
		Forest Soil Inoculum	1.5742	3.7894
		Control	1.8686	3.4965
	Cornus stolonifera	Mycopak	2.7457	5.8225
		Biogrow	2.7103	10.7067
		Forest Soil Inoculum	2.1748	3.4927
		Control	2.1918	4.0105
Total Powell & Stranahan	*Populus tremuloides*	Mycopak	1.6203	3.9472
		Biogrow	2.1116	4.8574
		Forest Soil Inoculum	2.0852	4.5864
		Control	1.8612	4.2542
	Salix bebbiana	Mycopak	1.0721	4.0615
		Biogrow	2.0724	10.7087
		Forest Soil Inoculum	1.2049	5.0657
		Control	.9208	4.3440
	Populus trichocarpa	Mycopak	1.3679	4.8236
		Biogrow	1.9974	4.4533
		Forest Soil Inoculum	1.4816	7.0746
		Control	1.5398	7.5285
	Cornus stolonifera	Mycopak	2.4404	5.6511
		Biogrow	2.5580	10.3855
		Forest Soil Inoculum	1.8224	4.6198
		Control	1.9327	5.9104

APPENDIX 5 – GLOSSARY

Ectomycorrhizae (EM) – A mycorrhizal association in which the fungal mycelia extend inward between root cortical cells to form a network ("Hartig net") and outward into the surrounding soil. Usually the fungal hyphae also form a mantle on the surface of the roots. A mycorrhizae that typically form between the roots of woody plants and fungi belonging to the divisions Basidiomycota, Ascomycota, or Zygomycota. (Found in 10% of plant families), mostly the woody species, including oak, pine, eucalyptus, dipterocarp, olive, and willow families.

Endomycorrhizae (arbuscular mycorrhiza) (AM) – A mycorrhizal association with intracellular penetration of the host root cortical cells by the fungus as well as outward extension into the surrounding soil. A mycorrhiza that involves entry of the hyphae into the plant cell walls to produce structures that are either balloon-like (vesicles) or dichotomously-branching invaginations (arbuscules).

Hyphae – A long branching filamentous cell of fungus. In fungi the hyphae are the main mode of vegetative growth. Collectively hyphae are referred to as mycelium. The hypha consists of one or more cells surrounded by a tubular cell wall. In most fungi, hyphae are divided into cells by internal cross-walls called septa, hence the term "septate hyphae."

Mycelium – The vegetative part of a fungus, consisting of a mass of branching, threadlike hyphae.

Mycorrhiza – Greek for fungus roots. A symbiotic, or sometimes weakly pathogenic association between a fungus and the roots of a plant. In a mycorrhizal association the fungus may colonize the roots of a host plant either intracellularly or extracellulary.

Propagule – Any plant material used for the purpose of plant propagation.

Tripartite – A system composed of or divided into three parts (seedling + AM fungi + EM fungi)